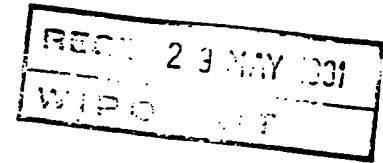AUSTRALIA

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**Patent Office
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I, RONALD MAXWELL MAY, ASSISTANT DIRECTOR PATENT
OPERATIONS, hereby certify that the annexed is a true
copy of the Provisional specification as lodged on 11
February 1991 in connection with Application No. PK 4537
for a patent by BIOTA SCIENTIFIC MANAGEMENT PTY LTD
lodged on 11 February 1991.

I further certify that pursuant to the provisions of
section 50(1) of the Patents Act 1952 a complete
specification was lodged on 24 April 1991 in respect of
Applications PJ 9800, PK 2896, PK 4537 and has been
allocated No. 75338/91.

I further certify that the annexed specification is not,
as yet, open to public inspection.

PRIORITY DOCUMENT

WITNESS my hand this  Seventeenth
day of May 1991.

RONALD MAXWELL MAY
ASSISTANT DIRECTOR PATENT OPERATIONS

APPLICANT:       BIOTA SCIENTIFIC MANAGEMENT PTY LTD

NUMBER:

FILING DATE:


## COMMONWEALTH OF AUSTRALIA

## The Patents Act 1952

PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

"ANTI-VIRAL COMPOUNDS"

This invention is described in the following statement:

## ANTI-VIRAL COMPOUNDS

5      This invention relates to a new class of
anti-viral compounds, including certain 2-deoxy and
2,3-dehydro analogues of $\kappa$-D-neuraminic acid, and to
their use, via inhibition of viral neuraminidases, for
the prophylaxis and for the treatment of infections such
10 as influenza, Newcastle disease, and fowl plague.

### Background of the Invention

Enzymes with the ability to cleave N-acetyl
neuraminic acid (NANA), also known as sialic acid, from
other sugars are present in many microorganisms. These
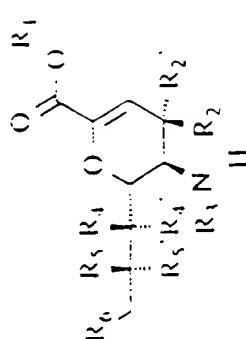15 include bacteria such as Vibrio cholerae, Clostridium

perfringens, Streptococcus pneumoniae, and Arthrobacter
sialophilus, and viruses such as influenza virus,
parainfluenza virus, mumps virus, Newcastle disease
virus, fowl plague virus, and Sendai virus.  Most of
5 these viruses are of the orthomyxovirus or paramyxovirus
groups, and carry a neuraminidase activity on the surface
of the virus particles.

Many of the neuraminidase-possessing organisms
are major pathogens of man and/or animals, and some, such
10 as influenza virus, Newcastle disease virus, and fowl
plague virus, cause diseases of enormous economic
importance.

It has long been thought that inhibitors of
neuraminidase activity could prevent infection by
15 neuraminidase-bearing viruses.  A variety of such
inhibitors is known;  most are analogues of neuraminic
acid, such as 2-deoxy-2,3-dehydro-N-acetylneuraminic acid
(DANA) and its derivatives (e.g. Meindl et al, Virology
1974 58 457-63), of which the most active is
20 2-deoxy-2,3-dehydro- N-trifluoracetylneuraminic acid
(FANA).  A number of such derivatives is known, and these
are summarized in Table 1.  FANA inhibits multi-cycle
replication of influenza and parainfluenza viruses
(Palese et al, Virology 1974 59 490-498).  Many of these
25 compounds are active against neuraminidase from V.
cholerae or Newcastle disease virus as well as that from
influenza virus.  Neuraminidase in at least some strains
of influenza or parainfluenza viruses is also inhibited
by 3-aza-2,3,4-trideoxy-4-oxo-D- arabinoctonic acid $S$
30 -lactone and
O- $\alpha$ -N-acetyl-D-neuraminosyl-(2 $\rightarrow$ 3)-2- acetamido-2-deoxy-
D-glucose (Zakstel'skaya et al. Vop. Virol. 1972 17
223-228).

## TABLE 1

### Known 2,3-dehydro derivatives on N-acetylneuraminic acid



| No. | $R_1$ | $R_2$ | $R_2'$ | $R_1'$ | $R_3$ | $R_4'$ | $R_5$ | $R_5'$ | $R_6$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | = | = | OH | CH₃CO- | = | OH | OH | = | OH |
| 2 | = | = | OH | NH₂CO- | = | OH | OH | = | OH |
| 3 | = | = | OH | HCO- | = | OH | OH | = | OH |
| 4 | = | = | OH | FCH₂CO- | = | OH | OH | = | OH |
| 5 | = | = | OH | F₂CHCO- | = | OH | OH | = | OH |
| 6 | = | = | OH | F₃CCO- | = | OH | OH | = | OH |
| 7 | = | = | OH | ClCH₂CO- | = | OH | OH | = | OH |
| 8 | = | = | OH | ICH₂CO- | = | OH | OH | = | OH |
| 9 | = | = | OH | CNCH₂CO- | = | OH | OH | = | OH |
| 10 | = | = | OH | NH₂CH₂CO- | = | OH | OH | = | OH |
| 11 | = | = | OH | HSCH₂CO- | = | OH | OH | = | OH |
| 12 | = | = | OH | CH₃CONHCH₂CO- | = | OH | OH | = | OH |
| 13 | = | = | OH | (CH₃)₂NCH₂CO- | = | OH | OH | = | OH |

## TABLE 1 (cont.)

| No. | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 |
|---|---|---|---|---|---|---|---|---|---|
| 14 | = | = | OH | $NH_2CH_2CH_2CO-$ | = | OH | OH | H | OH |
| 15 | = | = | OH | $CH_3CONHCH_2CHCO-$ | = | OH | OH | H | OH |
| 16 | = | = | OH | $HOOCCH_2CH_2CO-$ | = | OH | OH | = | OH |
| 17 | = | = | OH | $HOOCCH=CHCO-$ | = | OH | OH | = | OH |
| 18 | = | = | OH | $Neu5Acyl2enNHCOCH_2SCH_2CO-$ | = | OH | OH | = | OH |
| 19 | = | = | OH | $HOCH_2CO-$ | = | OH | OH | = | OH |
| 20 | = | = | OH | $CH_3CH_2CO-$ | = | OH | OH | = | OH |
| 21 | = | = | OH | $CH_3CH_2CH_2CO-$ | = | OH | OH | = | OH |
| 22 | = | = | OH | $C_6H_5CO-$ | = | OH | OH | = | OH |
| 23 | = | = | OH | $C_6H_5CH_2CO-$ | = | OH | OH | = | OH |
| 24 | $CH_3$ | = | OH | $CH_3CO-$ | = | OH | OH | = | OH |
| 25 | $CH_3$ | OH | = | $CH_3CO-$ | = | OH | OH | = | OH |
| 26 | $CH_3$ | = | OH | $CH_3CO-$ | = | OH | OH | = | OTr |
| 27 | $CH_3$ | OH | = | $CH_3CO-$ | = | OH | OH | = | $O(p\text{-}CH_3OC_6H_4)$ |
| 28 | $CH_3$ | = | OH | $CH_3CO-$ | = | OH | OH | = | $O(p\text{-}CH_3OC_6H_4)$ |
| 29 | $CH_3$ | $=O$ | | $CH_3CO-$ | = | OH | OH | = | $O(p\text{-}CH_3OC_6H_4)$ |
| 30 | $CH_3$ | $=O$ | | $CH_3CO-$ | = | OH | OH | = | OTr |
| 31 | $CH_3$ | $=O$ | | $CH_3CO-$ | = | OH | OH | = | OH |
| 32 | = | $=O$ | | $CH_3CO-$ | = | OH | OH | = | OH |

## TABLE 1 (cont.)

| | $R_1$ | $R_2$ | $R_2'$ | $R_3$ | $R_4$ | $R_3'$ | $R_5$ | $R_5'$ | $R_6$ |
|---|---|---|---|---|---|---|---|---|---|
| 33 | H | H | OH | CH₃CO- | H | H | OH | H | OH |
| 34 | H | H | OH | CH₃CO- | H | OH | H | H | OH |
| 35 | H | H | OH | CH₃CO- | H | OH | OH | H | H |
| 36 | H | H | H | CH₃CO- | H | H | OH | H | OH |
| 37 | CH₃ | H | CH₃COO- | CH₃CO- | H | H | CH₃COO- | H | CH₃COO- |
| 38 | CH₃ | H | CH₃COO- | CH₃CO- | H | CH₃COO- | H | H | CH₃COO- |
| 39 | CH₃ | H | CH₃COO- | CH₃CO- | H | CH₃COO- | CH₃COO- | H | H |
| 40 | CH₃ | H | H | CH₃CO- | H | H | CH₃COO- | H | CH₃COO- |
| 41 | CH₃ | H | C₆H₅CH₂O- | CH₃CO- | H | C₆H₅CH₂O- | C₆H₅CH₂O- | H | C₆H₅CH₂O- |
| 42 | CH₃ | H | CH₃COO- | CH₃CO- | H | CH₃COO- | CH₃COO- | H | CH₃COO- |
| 43 | CH₃ | H | CH₃COO- | CH₃CO | H | CH₃COO- | H | H | CH₃COO- |
| 44 | CH₃ | H | CH₃COO- | CH₃CO | H | CH₃COO- | H | CH₃COO- | H |
| 45 | CH₃ | H | CH₃COO- | CH₃CO | H | CH₃COO- | H | CH₃COO- | 2αNeu5Ac |

# TABLE 1 (cont.)
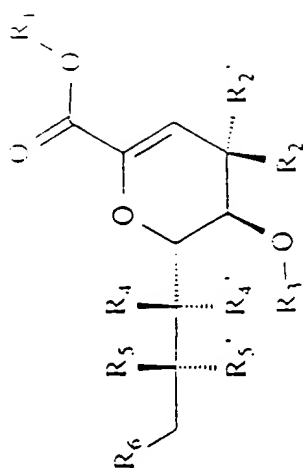


| | R_1 | R_2 | R_2' | R_3 | R_4 | R_4' | R_5 | R_5' | R_6 |
|---|---|---|---|---|---|---|---|---|---|
| 4ó | C_6H_5CH_2 | H | CH_3COO- | CH_3CO | H | CH_3COO- | CH_3COO- | H | CH_3COO- |

Compounds 1-18    P.Meindl, G. Bodo, P. Palese, J. Schulman and H. Tuppy. Inhibition of Neuraminidase Activity by Derivatives of 2-Deoxy-2,3.-dehydro-N-acetylneuraminic Acid. *Virology* 58, 457-463(1974).

Compounds 19-23    P.Meindl and H. Tuppy. Ueber 2-Desoxy-2,3-deshydro-sialinsaeuren 1. Mitt.: Synthese und Eigenschaften von 2-Desoxy-2,3-deshydro-N-acylneuraminsaeuren und deren Methylestern. *Mh. Chem.* 100 (4) 1295-1306 (1969)

Compounds 24-32    M. Flashner et al. Methyl-5-acetamido-2,6-anhydro-3,5-dideoxy-D-manno-non-2-en-4-ulosonate. *Carbohydrate Research* 103, 281-285(1982)

TABLE 1 (cont.)

| | |
|---|---|
| Compounds 33-40 | E. Zbiral et al. Synthesis of 2,7-, 2,8-, and 2,9-Dideoxy and 2,4,7-Trideoxy-2,3-didehydro-*N*-acetylneuraminic Acids and Their Behavior Towards Sialidase from *Vibrio cholerae*. *Liebigs Ann. Chem.* 1989, 159-165 |
| Compounds 41-42 | T. Ogawa and Y. Ito. An Efficient Approach to Stereo-selective Glycosylation of *N*-Acetylneuraminic Acid: Use of Phenylselenyl Group as a Stereocontrolling Auxillary. *Tetrahedron Letters* 28, (49), 6221-6224(1987). |
| Compounds 43-45 | T. Goto et al. Synthesis of ((α2-9) and (α2-8) Linked Neuraminylneuraminic Acid Derivatives. *Tetrahedron Letters* 27, (43), 5229-5232(1986). |
| Compound 46 | H. Ogura et al. Studies on Sialic Acids XV. Synthesis of α and β-*O*- Glycosides of 3-Deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic Acid (KDN). *Chem. Pharm. Bull* 36, (12), 4807-4813(1988) |

Neuraminidase from <u>Arthrobacter sialophilus</u> is inhibited by the glycals 2,3-dehydro-4-epi-N-acetyl-neuraminic acid, 2,3-dehydro-2-deoxy-N-acetylneuraminic acid, and 5-acetamido-2,6-anhydro-2,3,5-trideoxy-D-manno-
5 non-2-en-4-ulosonate, and by their methyl esters (Kumar et al Carbohydrate Res 1981 <u>94</u> 123-130; Kumar et al Carbohydrate Res 1982 <u>103</u> 281-285).

The thioanalogues 2-$\alpha$-azido-6-thio-neuraminic acid and 2,3-dehydro-6-thioneuraminic acid (Mack and
10 Brossmer: Tetrahedron Letters 1987 <u>28</u> 191-194) and the fluorinated analogue N-acetyl-2,3-difluoro-$\alpha$-D-neuraminic acid (Nakajima et al: Agric. Biol. Chem. 1988 <u>52</u> 1209-1215) were reported to inhibit neuraminidase, although the type of neuraminidase was not identified.
15 Schmid et al (Tetrahedron Letters 1958 <u>29</u> 3643-3646) described the synthesis of 2-deoxy-N-acetyl-$\alpha$-D-neuraminic acid, but did not report its activity or otherwise against neuraminidase.

Meindl and Tuppy (Hoppe-Seyler's Z. Physiol
20 Chem 1969, <u>350</u>, 1088) described hydrogenation of the olefinic double bond of 2-deoxy-2,3-dehydro-N-acetyl-neuraminic acid to produce the $\beta$-anomer of 2-deoxy-N-acetylneuraminic acid. This $\beta$-anomer did not inhibit <u>Vibrio cholerae</u> neuraminidase.
25 While these previously known inhibitors are competitive inhibitors of neuraminidases, none of the compounds is known to have anti-viral activity <u>in vivo</u>. Although Dernick et al (in Antiviral Chemotherapy ed. K.K. Gauri Academic Press 1981 p. 327-336), have asserted
30 that a half-planar, unsaturated 6-member ring system is important for inhibitory activity, some compounds, notably FANA characterized by such a system are reported to have no <u>in vivo</u> anti-viral activity, (Palese, P. and Schulman, J(1977) in Chemoprophylaxis and Virus Infection
35 of the Upper Respiratory Tract Vol 1 CRC Press ed. J.S. Oxford p. 189-205).

Thus conventional wisdom identifies the most potent in vitro inhibitors of viral neuraminidase as compounds that are based on the neuraminic acid framework, and these are thought by some to be

5 transition-state analogues (Miller, C.A., P. Wang, and M. Flashner (1978) Biochem. Biophys. Res. Comm. 83 1479).

We have now surprisingly found that, although FANA was found to be inactive in vivo (Palese and Schulman, op. cit.), DANA has high activity when

10 administered intranasally to mice. It appears that the dose, as well as the route of administration, may be crucial, because the compound is rapidly excreted when given by other routes (Nohle, U., J-M. Beau, and R. Schauer, (1982) Eur. J. Biochem. 126 543-548).

15 Summary of the Invention

It is therefore an object of the present invention to provide improved inhibitors of neuraminidase which have anti-viral activity in vivo.

It is also an object of the present invention

20 to provide medicinal compositions which can be used to prevent or ameliorate symptoms of viral infection.

It is a further object of the present invention to provide means for producing such medicinal compositions.

25 In achieving this object there has been provided, in accordance with one aspect of the invention, a biologically active substance that binds the active site ("receptor") of influenza virus neuraminidase such that said substance displays anti-orthomyxovirus or

30 paramyxovirus activity in an animal. In a preferred embodiment, the active substance displays:

(a) in vitro activity in an assay which detects binding of the active site of influenza virus neuraminidase; and

    (b)    in vivo anti-orthomyxovirus or
paramyxovirus activity.

    Preferably said in vivo activity is displayed
in mice challenged intranasally with influenza virus.

5     According to another aspect, the invention
provides a biologically active substance which possesses
stereochemical complementarity to an enzyme active site
comprised of amino acids positioned at atomic
co-ordinates enumerated as part of Figure 1 below, or a
10 subset thereof and said substance displays in vivo
activity against an orthomyxovirus or a paramyxovirus.
Preferably said stereochemical complementarity is such
that said compound has an a $K_i$ for said active site of
the less than $10^{-7}$M. More preferably said $K_i$ is less
15 than $0.5 \times 10^{-8}$M.

    Preferably according to either aspect the
substance is a carbohydrate comprising a non-
mutarotatable anomeric carbon atom. More preferably,
this carbon atom is optionally substituted by a
20 functional group. Even more preferably, said functional
group is carried on the $C_2$ carbon.

    In a preferred embodiment, the compound has
general formula I or general formula Ia:
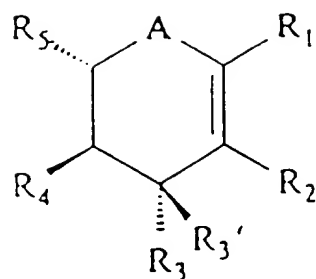
I
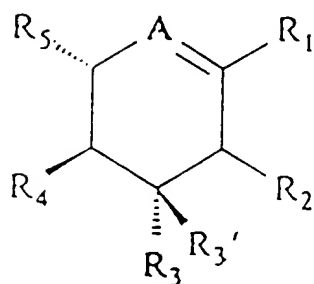


Ia

where in general formula I, A is oxygen, carbon
or sulphur, and in general formula Ia, A is nitrogen or
5 carbon;

$R^1$ denotes COOH, $P(O)(OH)_2$, $NO_2$, SOOH, $SO_3H$,
tetrazol, $CH_2CHO$, CHO, $CH(CHO)_2$ or, where $R^1$ is COOH,
$P(O)(OH)_2$, SOOH or $SO_3H$, an ethyl, methyl or pivaloyl
ester thereof,

10  $R^2$ denotes H, $OR^6$, F, Cl, Br, CN, $NHR^6$, $SR^6$ or
$CH_2X$, wherein X is $NHR^6$, halogen or $OR^6$ and

$R^6$ is hydrogen; an acyl group having 1 to 4
carbon atoms; a linear or cyclic alkyl group having 1 to
6 carbon atoms, or a halogen-substituted analogue

thereof; or an unsubstituted aryl group or an aryl substituted by a halogen, an OH group, and $NO_2$ group, and $NH_2$ group or a COOH group,

$R^3$ and $R^{3'}$ are the same or different, and each denotes hydrogen, CN, $NHR^6$, $N_3$, $SR^6$, =N-$OR^6$, $OR^6$, guanidino,
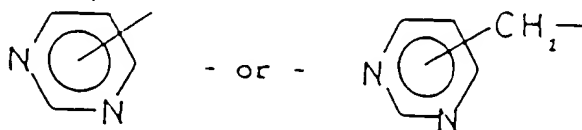


$R^4$ denotes $NHR^6$, $SR^6$, $OR^6$, $COOR^6$, $NO_2$, $C(R^6)_3$, $CH_2COOR^6$, $CH_2NO_2$ or $CH_2NHR^6$, and

$R_5$ denotes $CH_2YR^6$, $CHYR^6CH_2YR^6$ or $CHYR^6CHYR^6CH_2YR^6$, where Y is O, NH, S or H, and successive Y moieties in an $R^5$ group are the same or different,

and pharmaceutically acceptable salts or derivatives thereof.

In both these formulae $R^1$, $R^2$, $R^3$, $R^{3'}$, $R^4$, $R^5$ and $R^6$ are subject to the provisos that in general formula I,

(i) when $R^3$ or $R^{3'}$ is $OR^6$ or hydrogen, and A is oxygen or sulphur, then said compound cannot have both

(a) an $R^2$ that is hydrogen and

(b) an $R^4$ that is NH-acyl, and

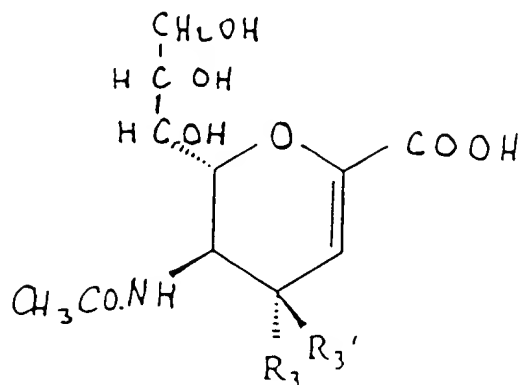(ii) $R^6$ represents a covalent bond when Y is hydrogen,

and that in general formula Ia,

(i) when $R^3$ or $R^{3'}$ is $OR^6$ or hydrogen, and A is nitrogen, then said compound cannot have both

(a) an $R^2$ that is hydrogen, and

(b) an $R^4$ that is NH-acyl, and

(ii) $R^6$ represents a covalent bond when Y is hydrogen.

In a more preferred embodiment, the compound has general formula II



5 i.e. in general formula I above, $R_1$ is COOH, $R_2$ is hydrogen, $R_4$ is acetamido, and $R_5$ is -CHOH.CHOH.CH$_2$OH, and $R_3$ is hydrogen or $R_3'$, where $R_3'$ denotes -N$_3$, -CN, -CH$_2$NH$_2$, or -N.R$_8$.R$_9$;

$R_8$ and $R_9$ are the same or different, and each
10 denotes hydrogen, a linear or cyclic alkyl group of 1 to
6 carbon atoms, an acyl or substituted acyl group of 1 to
6 carbon atoms, -C.(NH).NH$_2$, -CH$_2$.COOH, or -CH$_2$.CH.
$(R_9)(R_{10})$,

$R_9$ and $R_{10}$ may be the same or different, and
15 each denotes oxygen or $R_{11}$N=, and

$R_{11}$ denotes hydrogen, -OH, -OCH$_3$, -NH$_2$, or
$(CH_3)_2$N-.

In even more particularly preferred
embodiments, $R_3$ is selected from the group consisting of
20 amino, azido, and guanidino.

Most preferably the compound is selected from
the group consisting of

Sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-<u>glycero</u>-
D-<u>galacto</u>-non-2-enopyranosonate,
sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-<u>glycero</u>-
D-<u>galacto</u>-non-2-enopyranosonate, and
5 ammonium 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-
<u>glycero</u>-D-<u>galacto</u>-non-2-enopyranosate.

According to a second aspect, the invention
provides a pharmacologically active composition
comprising

10          (i)     an orthomyxovirus or
paramyxovirus-inhibiting amount of a substance that binds
the active site of influenza virus neuraminidase such
that said substance displays anti-orthomyxovirus or
paramyxovirus activity in an animal and

15          (ii)    a physiologically-compatible carrier
diluent or excipient for said substance.

Preferably the substance is a compound
according to general formula I, Ia, or II, but to which
the provisos set out above do not apply. More preferably

20 the substance is present at a concentration of 0.000001
to 100 mg/ml.

According to a third aspect, the invention
provides a method of preventing or ameliorating the
symptoms of an orthomyxovirus or paramyxovirus infection,

25 comprising the step of administering to an animal a
virus-inhibiting amount of a substance that binds the
active site of influenza virus neuraminidase such that
said substance displays anti-orthomyxovirus or
paramyxovirus activity in an animal.

30          In each of these three aspects of the
invention, the virus is preferably selected from the
group consisting of influenza virus, parainfluenza virus,
mumps virus, Newcastle disease virus, fowl plague virus,
and Sendai virus.

In the method according to the third aspect of
the invention, more preferably either the virus is
selected from the group consisting of influenza virus,
parainfluenza virus, Sendai virus and mumps virus, and
5 the animal is a human, or the virus is Newcastle disease
virus or fowl plague virus, and the animal is a bird.

The substance may be administered orally,
intranasally, buccally, or sublingually.

Preferably the substance is administered at a
10 dose of 0.0001 to 1000 mg/kg body weight.  The substance
is preferably as defined above.

Brief Description of the Drawings

Figures 1 and 2 represent reaction schemes for
the preparation of sodium 5-acetamido-4-azido-2,3,4,5-
15 tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate and,
the preparation of sodium 5-acetamido-4-amino-2,3,4,5-
tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate
respectively.

Detailed Description of the Preferred Embodiments
20 In general, the design of a molecule possessing
stereochemical complementarity can be accomplished by
means of techniques that optimize, either chemically or
geometrically, the "fit" between a molecule and a target
receptor.  Known techniques of this sort are reviewed by
25 Sheridan and Venkataraghavan (Acc. Chem Res. 1987, 20,
322), Goodford (J. Med. Chem. 1984, 27, 557), Beddell
(Chem. Soc. Reviews 1985, 279) and Hol (Angew. Chem.
1986, 25, 767), the respective contents of which are
hereby incorporated by reference.  See also Blundell et
30 al, Nature 1987, 326, 347 (drug development based on
information regarding receptor structure).

The use of a refined view of the
three-dimensional structure of the active site of
influenza virus neuraminidase, which we have developed

(with errors of only about 0.4 Å) is described in detail
in our U.S. Patent Application No. 423112 filed 19th
October 1990, the entire contents of which are herein
incorporated by reference.  This refined structure
5  enables the production of molecules which tightly bind
the enzyme active site, something that heretofore could
not have been accomplished based, for example, on extant
information regarding the crystal structure on N2
influenza virus neuraminidase soaked with neuraminic
10  acid.  See Varghese et al., Nature 303: 35-40 (1983).
Notwithstanding contrary expectations, discussed above,
as to the import of neuraminidase-binding capability, it
has also been discovered that compounds possessing high
affinity for the enzyme active site are also prime
15  candidates for in vivo anti-viral agents, which property
is routinely ascertainable by means of a conventional
animal assay, as described in greater detail below.

Thus, there are two preferred approaches to
designing a molecule, according to the present invention,
20  that complements the active site of influenza virus
neuraminidase.  By the geometric approach, the number of
internal degrees of freedom (and the corresponding local
minima in the molecular conformation space) is reduced by
considering only the geometric (hard-sphere) interactions
25  of two rigid bodies, where one body (the active site)
contains "pockets" or "grooves" that form binding sites
for the second body (the complementing molecule, as
ligand).  The second preferred approach entails an
assessment of the interaction of respective chemical
30  groups ("probes") with the active site at sample
positions within and around the site, resulting in an
array of energy values from which three-dimensional
contour surfaces at selected energy levels can be
generated.

The geometric approach is illustrated by Kuntz et al (J. Mol. Biol. 1982, 161, 269), the contents of which are hereby incorporated by reference, whose algorithm for ligand design is implemented in a
5 commercial software package distributed by the Regents of the University of California and further described in a document, provided by the distributor, which is entitled "Overview of the DOCK Package, Version 1.0,", the contents of which are hereby incorporated by reference.
10 Pursuant to the Kuntz algorithm, the shape of the cavity represented by the neuraminidase active site is defined as a series of overlapping spheres of different radii. One or more extant data bases of crystallographic data, such as the Cambridge Structural Database System
15 maintained by Cambridge University, (University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.) and the Protein Data Bank maintained by Brookhaven National Laboratory (Chemistry Dept. Upton, NY 11973, U.S.A.), is then searched for molecules which approximate the shape
20 thus defined.

Molecules identified in this way, on the basis of geometric parameters, can then be modified to satisfy criteria associated with chemical complementarity, such as hydrogen bonding, ionic interactions and Van der Waals
25 interactions. The chemical-probe approach to ligand design is described, for example, by Goodford (J. Med. Chem. 1985, 28, 849), the contents of which are hereby incorporated by reference, and is implemented in several commercial software packages, such as GRID (product of
30 Molecular Discovery Ltd., West Way House, Elms Parade, Oxford OX2 9LL, U.K.) Pursuant to this approach, the chemical prerequisites for a site-complementing molecule are identified at the outset, by probing the active site (as represented via the atomic coordinates shown in Fig.
35 1) with different chemical probes, e.g., water, a methyl group, an amine nitrogen, a carboxyl oxygen, and a

hydroxyl. Favoured sites for interaction between the active site and each probe are thus determined, and from the resulting three-dimensional pattern of such sites a putative complementary molecule can be generated.

5       The chemical-probe approach is especially useful in defining variants of a molecule known to bind the target receptor. Since sialic acid is such a molecule, vis-a-vis the neuraminidase active site, crystallographic analysis of sialic acid bound to
10 neuraminidase provides useful information regarding the interaction between an archetype ligand and the active site of interest. In particular, it has been found that sialic acid binds to neuraminidase in a distorted conformation, with the carboxylate group pushed into the
15 plane of the sugar.

       Since this carboxylate-planar feature is inherent in the DANA molecule and molecules that are "DANA-like" by virtue of having an $sp^2$-hybridized system at $C_2/C_3$, no distortion is needed for such molecules to
20 fit - that is, to possess stereochemical complementarity with relation to - the active site. The resulting increased complementarity of DANA and DANA-like molecules is reflected, for example, in respective $K_i$ values for DANA and a novel DANA derivative, 4-amino-2,3-dehydro-
25 2,4-dideoxy-N-acetylneuraminic acid, that are significantly lower (indicating higher active-site affinity) than the corresponding values for sialic acid and its derivatives. As described in greater detail below, the increased complementarity is also evidenced by
30 in vivo anti-viral activity of both DANA and the 4-amino DANA derivative, which was designed, according to the present invention, via the chemical-probe approach discussed above.

       Accordingly, a preferred subgroup of anti-viral
35 agents suitably used in pharmaceutical formulations of the present invention includes DANA-like molecules,

especially those with a $K_i$ of greater than $10^{-7}$. More
generally, 5-, 6- and 7-membered carbocyclic and
heterocyclic compounds that possess the structural
feature of carboxylate-planarity are preferred candidates
5 for anti-viral agents to use in accordance with the
present invention. Exemplary of such compounds are the
molecules represented, respectively, by formula I and
formula Ia. These molecules comprise a carboxylate
moiety that is positioned in the plane of the ring
10 nucleus by virtue of the $sp^2$-hybridized system which
includes the heteroatom or $C_3$, as the case may be, and
the carbon that bears the carboxylic-acid moiety or an
analogue thereof, where "analogue" denotes a moiety that
can interact either ionically (say, via hydrogen-bonding)
15 or covalently (via a Schiff reaction, for instance) with
a reactable amino moiety in the active site, such as is
presented by arginine 371 corresponding to the
coordinates for the atoms ARG NH1 371 and ARG NH2 371 of
the neuraminidase three-dimensional structure disclosed
20 in U.S. Patent Application No. 423112 referred to above.

It is known that single amino-acid changes can
cause major changes in activity of influenza virus
neuraminidase which are not predictable on the basis of
any theoretical method. Insofar as it may not be
25 necessary, for the complementarity between compound and
active site to extend over all residues of the active
site, compounds that bind atoms comprising fewer than all
of the residues of the active site are encompassed by the
present invention.

30 In summary, the general principles of
receptor-based drug design can be applied by persons
skilled in the art, using the crystallographic data
presented above, to produce compounds having sufficient
stereochemical complementarity to produce a high-affinity
35 binding of the active site of influenza virus
neuraminidase.

The present invention is further described below by reference to the following, non-limiting examples.

Example 1   The preparation of Sodium 5-Acetamido-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4)(4-Azido-Neu5Ac2en)

Designations of compounds are as in Figure 1.

Preparation of (2)

To an agitated solution of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonate (1)(1500 mg, 3.17 mmol) in a mixture of benzene (50 ml) and methanol (300 mg) was added dropwise $BF_3.Et_2O$ (12 ml) over thirty minutes under a nitrogen atmosphere at room temperature. The whole mixture was then allowed to stir at room temperature for 16 hours. The solution was diluted with ethyl acetate (250 ml), washed successively with saturated $NaHCO_3$ solution (30 ml x 3) and water (20 ml x 3), then evaporated to a small volume (about 10 ml), to which was added water (0.5 ml) and acetic acid (0. 5ml). The whole mixture was then stirred at room temperature for two days before being diluted with ethyl acetate (200 ml). The ethyl acetate solution was washed with 5% $NaHCO_3$ solution (30 ml x 2) and water (20 ml x 3), then evaporated to dryness. The residue was chromatographed (silica gel, ethyl acetate as eluting solvent) to afford pure compound (2) (550 mg, 40%).

1H-nmr ($CDCl_3$) $\delta$ (ppm); 1.95, 2.06, 2.08, 2.10, 2.35 (s, 15H, Acetyl $CH_3$ x 5), 3.80 (s, 3H, $COOCH_3$), 4.1-4.4 (m, 4H, $H_4$, $H_5$, $H_6$, $H_9$), 4.82 (dd, 1H, $J_{9,8}$ 1.8Hz, $J_{9,9'}$ 12.3Hz, $H_9$), 5.27 (m, 1H, $H_8$), 5.45 (bd, 1H, $J_{7,8}$ 3.5Hz, $H_7$), 6.15 (d, 1H, $J_{3,4}$ 5.4Hz, $H_3$), 6.47(d, 1H, $J_{NH,5}$ 3.8Hz, -CONH).

Preparation of (3)

To a stirred solution of compound (2) (800 mg, 1.67 mmol) in anhydrous dichloromethane (10 ml) and dry pyridine (316 mg, 4 mmol) at -30 to - 40°C, was added

5 dropwise a solution of trifluoromethane sulphonic anhydride (Tf$_2$O) (556 mg, 2 mmole) in dichloromethane (2 ml) over 15 minutes. The reaction mixture was then stirred at -30°C for 5 hours, and concentrated to dryness in vacuo. The residue was then dissolved in dry DMF (5

10 ml) containing a mixture of sodium azide (650 mg, 10 mmol) and tetrabutylammonium hydrogen sulphate (170 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 16 hours, and then evaporated to dryness under high vacuum. The residue was partitioned between

15 ethyl acetate (200 ml) and water (50 ml). The organic layer was separated and washed with water (50 ml x 2), dried over Na$_2$SO$_4$, evaporated to leave a residue (780 mg), which was subjected to double chromatography (silica gel, the first solvent system was ethyl acetate/acetone:

20 8/1; the second solvent system was dichloromethane/water: 10/1) to afford a colourless oil (3) (185 mg, 24%). MS. (FAB) 457 ($M^+$ + 1), 414($M^+$ - N$_3$. $[\alpha]^{20}_D$ + 19.1° (Cl, MeOH). i.r. (CHCl$_3$)cm$^{-1}$ 2100 (-N$_3$). 1748 (carbonyl). $^1$H-nmr (CDCl$_3$) $\delta$ (ppm). 2.04, 2.05, 2.06, 2.12, (s, 12H,

25 Acetyl CH$_3$ x 4). 3.79 (s, 3H, COOCH$_3$), 3.91 (ddd, 1H, $J_{5,NH}$ 8.4Hz, $J_{5,4}$ 8.8Hz, $J_{5,6}$9.9Hz, H$_5$), 4.17 (dd, 1H, $J_{9',8}$6.8Hz, $J_{9',9}$12.5Hz, H$_{9'}$), 4.42 (dd, 1H, $J_{4,3}$2.9Hz, $J_{4,5}$8.8Hz, H$_4$), 4.48 (dd, 1H, $J_{6,7}$2.3Hz, $J_{6,5}$9.9Hz, H$_6$), 4.64 (dd, 1H, $J_{9,8}$2.7Hz, $J_{9,9'}$12.5Hz, H$_9$), 5.31 (m, 1H,

30 $J_{8,7}$5.2Hz, $J_{8,9}$2.7Hz, $J_{8,9'}$6.8Hz, H$_8$), 5.45 (dd, 1H, $J_{7,6}$2.3Hz, $J_{7,8}$5.2Hz, H$_7$), 5.96 (d, 1H, $J_{3,4}$2.9Hz, H$_3$), 6.13 (d, 1H, $J_{NH,5}$8.4Hz, -CONH)

$^{13}$C-nmr (CDCl$_3$) $\delta$ (ppm)

20.7 (CH$_3$-CO-O-), 23.2 (CH$_3$CO-NH), 48.3 (C$_5$), 52.6
(COOCH$_3$), 57.8 (C$_4$), 62.1 (C$_9$), 67.7, 70.9 (C$_7$, C$_8$), 75.9
(C$_6$), 107.6 (C$_3$), 145.1 (C$_2$), 161.5 (C$_1$), 170.2, 180.3,
5 170.7, (acetyl -C = O x 4).


### Preparation of (4)

Compound (3) (50 mg, 0.11 mmol) was dissolved
in anhydrous methanol (5 ml) containing sodium methoxide
(8 mg, 0.15 mmol). The mixture was stirred at room
10 temperature for 2 hours and concentrated to dryness <u>in</u>
<u>vacuo</u>. The residue was taken up in water (3 ml), stirred
at room temperature for 1.5 hours, adjusted to pH 6-7
with Dowex 50 x 8 (H$^+$) resin, and then lyophilised to
afford the title compound (4) (35 mg, 94%).
15 i.r. (KBr)cm$^{-1}$ 3400 (br.-OH), 2100 (-N$_3$), 1714
(carbonyl).
$^1$H-nmr (D$_2$O) $\delta$ (ppm). 2.06 (s, 3H, acetyl CH$_3$), 3.64
(dd, 1H, $J_{9',8}$6.3Hz, $J_{9',9}$11.8Hz, H$_{9'}$), 3.65 (dd, 1H,
$J_{7,6}$3.9Hz, $J_{7,8}$6.8Hz, H$_7$), 3.88 (dd, 1H, $J_{9,8}$2.6Hz,
20 $J_{9,9'}$11.8Hz, H$_9$), 3.94 (m, 1H, $J_{8,7}$6.8Hz, $J_{8,9}$2.6Hz,
$J_{8,9'}$6.3Hz, H$_8$), 4.21 (dd, 1H, $J_{5,4}$10.4Hz, $J_{5,6}$8.9Hz,
H$_5$), 4.31 (dd, 1H, $J_{4,3}$2.2Hz, $J_{4,5}$2.2Hz, $J_{4,5}$10.4Hz, H$_4$),
4.34 (dd, 1H, $J_{6,5}$8.9Hz, $J_{6,7}$3.9Hz, H$_6$) 5.82 (d, 1H,
$J_{3,4}$2.2Hz, H$_3$).


25 Example 2  The preparation of Sodium 5-Acetamido-4-
amino-2,3,4,5-tetradeoxy-D-glycero-
D-galacto-non-2-enopyranosonate (6)(4-
amino-Neu5Ac2en)

Designations of compounds are as in Figure 2.
30  Preparation of (5)

Into a solution of methyl
5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-
D-glycero-D-galacto-non-2-enopyranosonate (3) (95 mg,
0.208 mmol) in pyridine (6 ml) was bubbled H$_2$S for 16

hours at room temperature. The solution was then flushed with nitrogen for 15 minutes, and evaporated to remove pyridine under high vacuum. The residue was chromatographed (silica gel, ethyl acetate/iso-propanol/

5  water = 5/2/1) to afford a colourless compound (5) (50 mg, 56%).

MS. (FAB) 431 ($M^+$ + 1), 414 ($M^+$ -NH$_2$), $[\alpha]^{20}_D$ + 34.5° (Cl, MeOH). i.r. (CHCl$_3$) Cm$^{-1}$ 3400 (br. NH$_2$), 1740 (carbonyl).

10 $^1$H-nmr (CDCl$_3$ + CD$_3$OD) $\delta$(ppm). 1.96, 2.06, 2.07, 2.10 (s, 12H acetyl CH$_3$ x 4), 3.81 (s, 3H, -COOCH$_3$), 3.92 (brt, 1H, $J_{5,4}$ & $J_{5,6}$, 10Hz, H$_5$), 4.17 (dd, 1H, $J_{9',8}$7.2Hz, $J_{9',9}$12.3Hz, H$_9$,), 4.22 (br. dd, 2H, $J_{4,5}$ & $J_{6,5}$10Hz, $J_{4,3}$ & $J_{6,7}$2.1Hz, H$_4$ & H$_6$), 4.71 (dd, 1H, $J_{9,8}$2.6Hz,

15 $J_{9,9'}$12.3Hz, H$_9$), 5.31 (m, 1H, $J_{8,7}$4.9Hz, $J_{8,9}$2.6Hz, $J_{8,9'}$7.2Hz, H$_8$), 5.45 (d, 1H, $J_{7,6}$2.1Hz, $J_{7,8}$4.9Hz, H$_7$), 5.97 (d, 1H, $J_{3,4}$2.1Hz, H$_3$).

$^{13}$C-nmr (CDCl$_3$ + CD$_3$OD $\delta$(ppm)
20.2, 20.3 (CH$_3$-CO-O-), 22.3 (CH$_3$-CO-NH), 48.2(C$_5$),

20 50.4(C$_4$), 52.0 (COOCH$_3$), 62.1(C$_9$, 67.8, 71.2 (C$_7$, C$_8$, 76.5(C$_6$), 112.5(C$_3$), 143.6(C$_2$), 162.0(C$_1$), 170.2, 170.4, 170.8, 172.2. (acetyl -C = O x 4).

### Preparation of (6)

Compound (5) (50 mg, 0.116 mmol) was dissolved

25 in anhydrous methanol (5 ml) containing sodium methoxide (12.4 mg, 0.23 mmol). The mixture was stirred at room temperature for 1.5 hours and evaporated to dryness in vacuo at 30°C. The residue was stirred in water (3 ml) at room temperature until TLC (silica gel, ethyl

30 acetate/methanol/0.1 N HCl = 5/4/1) indicated that hydrolysis was complete. The solution (pH - about 10.5) was then gradually adjusted to around pH 7.5 by Dowes 50 x 8 (H$^+$) resin. As soon as the pH of the solution

reached 7.5, the suspension was quickly filtered by a
press filter.  The filtrate was lyophilised to afford the
title compound (6) (30 mg, 83%).

$^{1}$H-nmr (D$_2$O) $\delta$ (ppm).  2.07 (s, 3H, acetyl CH$_3$), 3.59 -
5 3.70 m, 2H, H$_7$ & H$_{9'}$), 3.89 (dd, 1H J$_{9,8}$2.6Hz,
J$_{9,9'}$11.8Hz, H$_9$), 3.95 (m, 1H, H$_8$), 3.99 (brd, 1H,
J$_{4,5}$10.6Hz, H$_4$), 4.21 (brt, 1H, J$_{5,4}$ & J$_{5,6}$10.6Hz, H$_5$),
4.29 (brd, 1H, J$_{6,5}$10.6Hz, H$_6$), 5.66 (d, 1H J$_{3,4}$1.9Hz,
H$_3$).

10 <u>Example 3</u>    <u>Inhibition of influenza virus neuraminidase</u>
        <u>In vitro</u> bioassay of compounds against N2
influenza virus neuraminidase followed the same protocol
as that developed by Warner and O'Brien (Biochemistry,
1979 <u>18</u> 2783-2787).   For comparison, using the same assay
15 it was found that K$_i$ for 2-deoxy-N-acetyl-$\alpha$-D-neuraminic
acid was 3 X 10$^{-4}$ M.

        K$_i$ values were determined using a
spectrofluorometric technique using the fluorogenic
substrate 4-methylumbelliferyl N-acetylneuraminic acid
20 (MUN) described by Meyers et al. (Anal. Biochem. <u>101</u>,
166-174 (1980).  For both enzymes, the assay mixture
contained test compound at several concentrations between
0 and 2 mM, and approximately 1 mU enzyme in buffer (32.5
mM MES, 4 mM CaCl$_2$, pH 6.5 for N2; 32.5mM Acetate, 4 mM
25 CaCl$_2$, pH  5.5 for <u>V. cholerae</u> neuraminidase).  The
reaction was started by the addition of MUN to final
concentrations of 75 or 40 uM.  After 5 minutes at 37$^{O}$C,
2.4 ml 0.1 M Glycine-NaOH, pH 10.2 was added to 0.1 ml
reaction mixture to terminate the reaction.  Fluorescence
30 was read at excitation 365 nm; emission 450 nm, and
appropriate MUN blanks (containing no enzyme) were
subtracted from readings.  The K$_i$ was estimated by Dixon
plots (1/Fluorescence versus Compound concentration).
Results are summarized in Table 2.

Table 2


Inhibition of influenza virus neuraminidase <u>in vitro</u>


| Compounds | $K_i$ (M) |
|---|---|
| 2-deoxy-N-acetyl-α-D-neuraminic acid | $3 \times 10^{-4}$ |

5  Sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy- $2.0 \times 10^{-6}$
  D-<u>glycero</u>-D-<u>galacto</u>-non-2-enopyranosonate


  Sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy- $4.0 \times 10^{-8}$
  D-<u>glycero</u>-D-<u>galacto</u>-non-2-enopyranosonate    $1.90 \times 10^{-7}$

(N9 neuraminidase,

10                                        pH 6.5)

$1.00 \times 10^{-8}$

(N2 virus,

pH 7.5)


    Results are for N2 neuraminidase at pH 6.5, unless
15 otherwise indicated.


    N2 virus:   detergent-inactivated whole virus.

Example 4    Preparation and activity of
Ammonium 5-acetamido-4-guanidino-2,3,4,5-
tetra-deoxy-D-glycero-D-galacto-non-2-
enopyranosate (7)
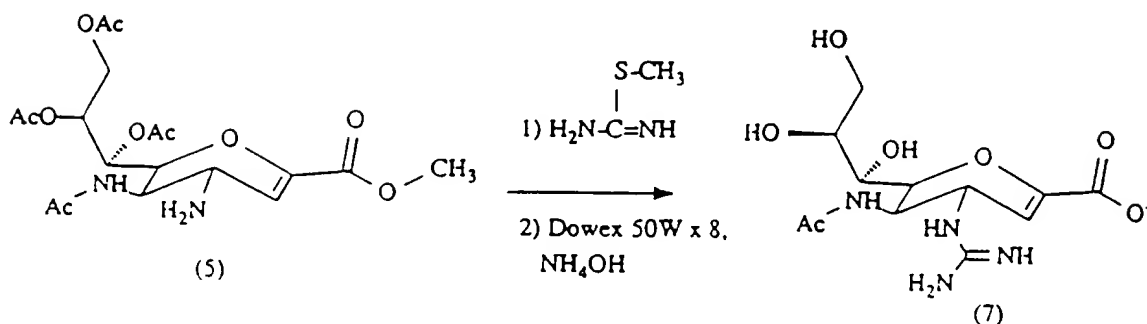


5 Methyl 5,7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-
glycero-D-galacto-non-2-enopyranosate (5)(40mg,
0.093mmol) was added into a solution of S-methylisourea
(546mg, 3mmol) in water (15mL) at ice-bath temperature.
The reaction mixture was stirred at 5°C for seven days,
10 then poured onto a column of Dowex 50W8(H+) resin (35mL),
washed with cold water (700mL), and eluted with 1.5M
$NH_4OH$ solution.  The eluate (120mL) was concentrated to
dryness under high vacuum.  The residue was then
chromatographed (silica gel, solvent system 1:ethyl
15 acetate/isopropanol/water, 1/5/1; solvent system 2:75%
isopropanol) to afford the title compound (7)(8mg, 24.5%)
$^1$H-nmr($D_2O+CD_3OD$) $\delta$ (ppm).
2.06(s, 2H, acetyl $CH_3$), 3, 60(br.d., 1H, $J_{7,8}$ 9.4 Hz,
$H_7$), 3.63(dd, 1H, $J_{9',8}$ 2.6 Hz, $J_{9',9}$ 11.8 Hz, $H_{9'}$), 3.76
20 (br.d., 1H, $J_{4,5}$ 9.4 Hz, $H_4$), 3.87 (dd, 1H, $J_{9,8}$ 2.6 Hz,
$J_{9,9'}$ 11.8 Hz, $H_9$), 3.93 (ddd, 1H, $J_{8,7}$ 9.4 Hz, $J_{8,9}$ 2.6

Hz, $J_{8,9}$, 6.2 Hz, $H_8$), 4.01 (dd, 1H, $J_{5,4}$ 9.4 Hz, $J_{5,6}$ 10.6 Hz, $H_5$), 4.20 (br.d., 1H, $J_{6,5}$ 10.6 Hz, $H_6$), 5.63 (d, 1H, $J_{3,4}$ 2.1 Hz, $H_3$).

A strong positive Sakaguchi reaction was given
5 by compound (7), indicating the presence of a guanidine group. Compound (7) was found to be a competitive inhibitor of influenza virus neuraminidase and of other neuraminidases. Results obtained using the in vitro assay described in Example 3 above are presented in Table
10 3.

## TABLE 3

| $K_i$ (M) | Neuraminidase |
|---|---|
| $1.7 \times 10^{-8}$ (at pH 6.5) | N2 virus |
| $6 \times 10^{-9}$ (at pH 7.5) | N2 |
| $\geqslant 5 \times 10^{-8}$ (at pH 6.5) | N9 |
| $4.5 \times 10^{-4}$ (at pH 5.8) | Vibrio cholerae |
| $> 10^{-2}$ (at pH 4.5) | Sheep (partially purified liver extract) |

15

### Example 5    In vivo anti-viral activity

20        The 4-amino compound of Example 2, which was shown in Example 4 to have anti-neuraminidase activity in vitro, was tested for anti-viral activity in vivo, using a standard type of assay.

When administered intranasally to mice before
25 and during challenge with influenza A virus, this compound reduced the titre of virus in lung tissue 1 to 3 days after infection.

Mice were infected intranasally with 50 μl of $10^3$ TCID$_{50}$ units/mouse of H2N2 influenza A virus
30 (A/Sing/1/57). The compound was administered intranasally at a dose rate of either 25 mg/kg body weight or 100 mg/kg body weight (50 μl of aqueous

solution/mouse) as follows:    24 hours and 3 hours before
infection; 3 hours after infection; then twice daily on
each of days 1, 2 and 3 after infection.

      The mice were sacrificed on days 1, 2 and 3
5 after infection, their lungs removed and virus titres in
the lungs measured.   The titres were plotted graphically
and expressed as the areas under the curves (AUC).

      The compound showed similar potency to DANA
when given intranasally to mice at a single dose level of
10 25 mg/kg body weight,


## Pharmaceutical Compositions

      A pharmaceutical formulation within the present
invention combines, with an active agent that binds the
viral neuraminidase active site and displays _in vivo_
15 anti-viral activity, a carrier for the active agent which
is pharmaceutically acceptable.   A pharmaceutically
acceptable carrier is a solid, liquid or gaseous material
that can be used as a vehicle for administering a
medicament because the material is inert or otherwise
20 medically acceptable, as well as compatible with the
active agent, in a particular context of administration.
In addition to a suitable excipient, a pharmaceutically
acceptable carrier can contain conventional additives lke
diluents, adjuvants, antioxidants, dispersing agents and
25 emulsifiers, anti-foaming agents, flavour correctants,
preservatives, solubilizing agents and colourants.

      The nature of the excipient used with an
anti-viral agent, pursuant to the present invention, is
largely a function of the chosen route of administration,
30 as discussed, for example, in REMINGTON'S PHARMACEUTICAL
SCIENCES, E.W. Martin (ed.), and in PHARMACEUTICAL DOSAGE
FORMS AND THEIR USE, H. Hess (ed.) (Hans Huber Publ.
1985), the respective contents of which are hereby
incorporated by reference.   Preferably, the
35 pharmaceutical compositions of the present invention are

provided in a unitary-dosage form which is suitable for administration intranasally, orally, buccally or sublingually.

In accordance with the present invention, a pharmaceutical composition is advantageously delivered to the throat, nasal cavity or lungs, the intranasal route of administration being especially preferred. Delivery of an active agent to the nasal cavity can be achieved with preparations of the present invention that take the form, for example, of an aerosol or vapour, a nasal spray or nose drops, or an inhalation powder. For these applications, it may be appropriate for the active agent to be micronized, for example, to a particle size on the order of 5 microns or less.

Suitable means for effecting delivery by direct application to the mucosal lining or via inhalation are well known to the art, for example, in the context of treating asthma. In this category are squeeze-bottle devices (nebulisers) and pressurized packs, for delivering a solution of the active agent as a spray into the nose, and conventional insufflators like the Spinhaler turbo-inhaler and liquid aerosol "puffers" (Spinhaler is a registered trade mark of Fisons Corporation), which deliver metered doses of a pharmaceutical preparation.

If the active agent is delivered from solution, as would typically be the case for a nasal spray or nose drops, the carrier preferably comprises distilled water that is both sterile and substantially free of fever-inducing (pyrogenic) substances, thereby to minimise the incidence of medical complications relating to contamination. Suitable propellants to comprise carriers for use in administration by pressurized aerosol are well known, including halogenated fluorocarbon gases, carbon dioxide, and nitrogen. See, e.g., Lachman et al. in THE THEORY AND PRACTICE OF INDUSTRIAL PHARMACY (Lea

and Febiger, Philadelphia 1976). In addition, a carrier
for administration via intranasal delivery or
insufflation may contain a pharmaceutically acceptable
surface-active agent, such as a fatty acid like oleic
5 acid or a detergent like Tween 80 or Span 80, in order to
enhance uptake of the active agent.

Conventional forms which are favoured for oral
administration include lozenges and pastilles, sublingual
and buccal tablets, and oral sprays. Numerous carriers
10 suitable for these forms are known, including solid
pulverulent carriers comprising a simple sugar or
corresponding alcohol (lactose, saccharose, sorbitol,
mannitol, etc), a starch such as potato starch, corn
starch or amylopectin, cyclodextrin, a cellulose
15 derivative, and gelatine. Liquid carriers can also be
employed to form suspensions, syrups, elixirs and
solutions containing the active agent.

In formulating a pharmaceutical preparation of
the present invention for oral administration, a solid
20 carrier would typically be mixed with a lubricant, such
as magnesium stearate, calcium stearate or a polyethylene
glycol wax, and then compressed into tablet form. In
keeping with common practice, tablets can be coated with
a concentrated sugar solution which may contain
25 components like gum arabic, gelatine, talcum and titanium
dioxide. Alternatively, tablets can be coated with a
lacquer dissolved in a readily volatile organic solvent.

A pharmaceutical composition within the present
invention contains a virus-inhibiting amount of an active
30 agent as described above. The optimum dosage of the
active compound will vary with the particular case, and
can be determined routinely in the clinical context,
which may be prophylactic or therapeutic. 'Prophylactic'
treatment is to be understood to mean treatment intended
35 to prevent or retard second-cycle infection as defined
below, thus preventing the establishment of the complete

clinical manifestations of the disease caused by that virus. 'Therapeutic' treatment is to be understood to mean treatment intended to alleviate the symptoms and severity of infection which is already established, by

5  disrupting release of virus particles and thus preventing further cycles of viral replication. Generally, the amount of active agent present in a pharmaceutical composition of the present invention should be sufficient to inhibit at least second-cycle infection by

10  orthomyxovirus or paramyxovirus in an animal. That is, an initial viral infection of a cell culminates in the assembly and budding of virus particles at the cell-membrane surface, which would be followed in the normal course by release of the particles and infection

15  thereby ("second-cycle infection") of other cells. A suitable amount of active agent to include in a pharmaceutical composition of the present invention would thus retard at least this second cycle of infection by virus, it is thought by inhibiting the action of

20  neuraminidase that results in release of virus particles from the membrane surface.


The composition may suitably be administered a few times daily at a dose level of about 0.0001 mg to 1000 mg per kg body weight.

25      It will be clearly understood that the invention in its general aspects is not limited to the specific details referred to hereinabove.


DATED this 11th day of February 1991

BIOTA SCIENTIFIC MANAGEMENT PTY LTD
By Their Patent Attorneys:

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# PATENT COOPERATION TREATY

## PCT

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| International publication No. | Addressee's file reference |
|---|---|
| WO91/16320  PCT/AU91/00161 | |

Pursuant to the addressee's request of ___26 February 1997___ ,

1. [X] the International Bureau hereby transmits a copy of the following application(s), the priority of which was claimed in the international application:

| country | date | number |
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| AU | 24.04.91 | PJ 9800 |
| AU | 19.10.90 | PK 2896 |
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